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**Biological impact of xeno-free chemically defined cryopreservation medium on amniotic epithelial cells.**

**Journal:** Stem Cell Res Ther

**Publication Year:** 2016

**Authors:** Toshio Miki, Wisia Wong, Elton Zhou, Anthony Gonzalez, Irving Garcia, Brendan H Grubbs

**PubMed link:** 26758986

**Funding Grants:** Generation of hepatic cell from placental stem cell for congenital metabolic disorders

**Public Summary:**

In this original manuscript, we describe a study on animal product-free chemically defined media specialized to freeze down (cryopreserve) amniotic epithelial cells. This is an essential study to establish a standardized protocol for therapeutic amnion-derived stem cell banking. We observed differences in the expression of stem cell marker genes (OCT4, SOX2, and NANOG) and stem cell surface markers (TRA1-60 and HESCA-1) following cryopreservation in different tested media. To the best of our knowledge, this is the first report that demonstrates that some commercially available freezing media are better able to preserve stem cell populations of primary human amniotic epithelial cells when compared to others, and, allow for improved maintenance of stem cell characteristics.

**Scientific Abstract:**

**BACKGROUND:** Amnion-derived stem cells have been proposed for cell replacement therapy and tissue regeneration. An easily accessible cell source, the placenta, allows us to potentially establish a bio-bank of cells for immunotype matched clinical applications. Several xeno-free (XF) cryopreservation media are currently available for pluripotent stem cells, however, these media have not yet been evaluated for the cryopreservation of amnion-derived stem cells. **METHODS:** Human amniotic epithelial cells were collected using standard protocols, and stored at -160 degrees C in one of five commercially available media. Cells frozen in standard media containing fetal bovine serum served as controls. Cells were then thawed, and evaluated for viability, mitochondrial membrane stability, and senescence status. Quantitative real time PCR was utilized to assess for expression of stem cell genes, and flow cytometry was used to identify the stem cell surface markers. **RESULTS:** Cell recovery and repopulation assays indicated no significant difference between XF media versus standard cryopreservation medium. In addition, no impact was observed on the senescence status, the cytostructural or mitochondrial morphology between the tested cryopreservation media. Differences were observed on the expression of stem cell marker genes (OCT4, SOX2, and NANOG) and a cell surface marker (TRA1-60) following cryopreservation in different chemically defined XF media, however, these were not statistically significant. **CONCLUSIONS:** Xeno-free cryopreservation of human amnion-derived stem cells is feasible and can be standardized to establish a bio-bank with human amnion-derived stem cells for future clinical application. Optimization of this media may allow for improved preservation of stem cell-like characteristics.

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